REMARKS

Claims 1 and 3 have been amended to address the rejections posed in the Office Action dated October 30, 2008. New claims 4-8, which are supported by the specification, have been added. Applicants submit that no new matter is added.

The Office Action rejected claims 1-3 over Erices *et al.* (British Journal of Haematology 2000, 109: 235-242), in view of Nishikawa *et al.* (publ. no. US 2004/0235160), and further in view of Petaja *et al.* (Journal of Clinical Investigation 1997, 99(11): 2655-2663). Applicants respectfully traverse.

The present invention is directed to a method of isolation of mesenchymal stem cells (MSCs) from umbilical cord blood (UCB). Since none of the references, whether taken alone or combined, disclose or teach the claimed isolation method, the Office Action does not establish *prima facie* obviousness. Further, the combination asserted by the Office Action does not render the claims obvious because the references would not be combined as set forth and, even if combined, there would be no reasonable expectation of success. Accordingly, the rejections should be withdrawn. The remarks presented below further present additional arguments.

Erices *et al.* reference:

The Office Action incorrectly states that the Applicant differentiates the Erices *et al.* reference from the present invention on the basis of mesenchymal progenitor cells (MPCs) being isolated from UCB in the former compared to the isolation of MSCs from UCB in the latter. Further, it is incorrect that the difference is merely in the specifics of dilution or culturing media. Instead, there are significant differences between Erices *et al.* and the present invention, even in view of the secondary references, as discussed below.

Erices *et al.* isolate MPCs from UCB via a method that is conventionally used for isolation of bone marrow cells (see Majumdar *et al.*, Journal of Cell Physiology 1998, 176(1): 57-66). Erices *et al.* does not disclose or use a medium that includes Stem Cell

Factor, GM-CSF (granulocyte-macrophage colony-stimulating factor), G-CSF (granulocyte colony-stimulating factor), IL-3 (interleukin-3), and IL-6 (interleukin-6) as required by claim 1. As discussed further below, the secondary references do not overcome this omission.

Further, as set forth in the prior response, the Office Action does not adequately or correctly consider the state of the art that describes the need for methods to isolate MSCs from UCB. The state of the art teaches that one would *not* expect to isolate MSCs from UCB and, at the very least, severely limits the scope of the Erices *et al.* method. For example:

- Mareschi *et al.* (Haematologica 2001, 86: 1099-1100) state that "our results suggest that early fetal blood is rich in MSCs but [full] term UCB is not" (Abstract, Mareschi *et al.*) and "Erices *et al.* (2000) showed that MSCs could be separated from UCB but this was mainly from *preterm* UCB" (emphasis added, page 124, right column, Mareschi *et al.*). This casts significant doubt on the rate of success Erices *et al.* achieve with their methods for isolating MSCs from UCB. Further, Mareschi *et al.* notes that isolating MSCs from UCB has not been accomplished by the Erices *et al.* group since UCB is a source of hematopoietic stem cells (HSCs) only: "it was possible to isolate MSCs from bone marrow but not from UCB" and "BM [bone marrow] contained mesenchymal stem cells that could easily be expanded and induced to differentiate for therapeutic use while the UCB adherent monolayer displayed the morphology and the characteristics of hematopoietic cells and not those of mesenchymal cells" (page 1099, Mareschi *et al.*).
- Romanov *et al.* (Stem Cells 2003, 21: 105-110) indicates that Erices *et al.* may not have isolated mesenchymal cells via the method disclosed since "umbilical cord blood is a rich source of hematopoietic stem/progenitor cells and does not contain mesenchymal progenitors" (Abstract). Romanov *et al.* isolated MSCs from a different source than the present invention since they "attempt[ed] to isolate MSCs from the *subendothelial layer* of umbilical cord vein" (emphasis added, Abstract, Romanov *et al.*) and did not isolate MSCs from umbilical cord *blood* as in the present invention.
- Wexler *et al.* (British Journal of Haematology 2003, 121: 368-374) state that "adult

BM [bone marrow] is a reliable source of functional cultured MSC, but CB [umbilical cord blood] and PBSC [peripheral blood stem cell collections] are not" (page 368, Wexler *et al.*). Further, the MSC-like cell type of Wexler *et al.* isolated had a $CD45^+$ phenotype that is inconsistent with MSCs: "CB [umbilical cord blood] and PBSC [peripheral blood stem cell collections] mononuclear cells cultured in MSC conditions for two passages produced a population of adherent, non-confluent fibroblast-like cells with a *haemopoietic phenotype, CD45*$^+$, $CD14^+$, $CD34^-$, $CD44^-$ and $CD29^-$" (emphasis added, Abstract, Wexler *et al.*). Notably, the MSCs of the present invention are *true* CD45-negative MSCs that do not have the haemotopoietic $CD45^+$ phenotype (Table 1 of the present application): "It was confirmed from Table 1 that in the case of stem cells isolated and cultured in accordance with the present invention, CD34, CD45 and CD14, which are characteristic indicators of hematopoietic stem cells showed negative reactions, [while] SH2, SH3, CD29 and CD44, which are characteristic indicator[s] of mesenchymal stem cells, showed positive reactions..." (paragraph 0026, present application).

MSC-like cells isolated with difficulty from CB cannot be comparable to the improvement in the field as disclosed by the claims of the present invention, whereby *actual* MSCs are isolated *reliably* from UCB. Further, as shown in Erices *et al.* itself, MSCs were isolated from pre-term UCB. In contrast, the present invention is useful in isolating MSCs from full-term UCB. Applicants' method as detailed in the claims successfully isolates MSCs from full-term UCB reliably at a rate of 98% compared to 2% in the field (Table 2 of the present application). Thus, the differences in methods between the present invention, Erices *et al.*, and the other prior methods is significant in allowing for the isolation of MSCs from UCB.

Nishikawa *et al.* reference:

Nishikawa *et al.* does not cure the deficiencies of Erices *et al.* as indicated above. The Office Action states that Nishikawa *et al.* is cited only to show the presence of additional components in the culture media. However, a disclosure that some culture

media contain additional components does not, by itself, make the reference combinable with Erices *et al.* in a manner that renders the claims obvious. A proper analysis of Nishikawa *et al.* reveals that one skilled in the art would not be motivated to combine Nishikawa *et al.* (which relates to HSCs) with Erices *et al.* (which related to MSCs) as outlined in the Office Action. Further, even if one combined these references, there would be no reasonable likelihood of success (see MPEP 2143.01 section III: "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art."; and 2143.01 section V: "If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification.").

A person of ordinary skill in the art would recognize that different additives and cytokines are utilized to grow various types of cells and to differentiate cells into specific cell types. Nishikawa *et al.* utilizes a mixture containing a particular stimulating factor, gp130, to specifically derive the HSC population from a culture. Not only does the present invention not utilize this stimulating factor, but using gp130 would limit the cells isolated to be exclusively HSCs, which are "cell[s] having the ability to differentiate into all lineages of the *blood cells*" (emphasis added, paragraph 0021, Nishikawa *et al.*) and are a less diverse cell type than the pluripotent MSCs. A person seeking to isolate MSCs would, therefore, not look to Nishikawa *et al.* in seeking to improve the Erices *et al.* method.

Nishikawa *et al.* "utilize stromal cells [as an additive] which provide a suitable environment for the maintenance and expansion of hematopoietic cells to support the maintenance and expansion of hematopoietic stem cells without differentiation" (paragraph 00006, Nishikawa *et al.*). Thus, the supplements utilized by Nishikawa *et al.* are unlike those of the present invention, which does not utilize these feeder systems. Further, the MSCs used in Example 5 of Nishikawa *et al.* are "harvested and cultured from normal human bone marrow" and not UCB as in the present invention. Additionally, these supplements are used for culturing HSCs not MSCs as in the present invention. Thus, using gp130 (which is essential to the teaching of Nishikawa) in the present invention is not possible because that would lead to stimulating alternate

differentiation pathways resulting in the isolation of a more limited cell type, the HSCs (which only differentiate in blood cells), rather than the MSCs, which are "primitive cells that are able to differentiate into bone, cartilage, adipose tissue, nerve, and muscle" (lines 15-16, present application). Thus, if one combined the teaching of Nishikawa *et al.* with Erices *et al.*, the modification would be unsatisfactory for the isolation of MSCs and, in fact, would not work for its intended purpose.

Petaja *et al.* and further matters:

The Office Action mentions the Petaja *et al.* reference that discusses "anticoagulant synergism of heparin and activated protein C *in vitro*" (Title, Petaja *et al.*). This reference does not discuss isolating stem cells and is not relevant to the present invention. As such, there is no motivation to combine this reference with Erices *et al.* or Nishikawa *et al.* to arrive at the present invention.

The Office Action indicated that claim 1 unconventionally refers to "45 ml per unit". However, in claim 1, "unit" does not mean anti-coagulant unit but means cord blood unit, which is a well-known term utilized in the art (refer to paragraph 0011 of the present invention). In this sense, "unit" refers to an individual *collection* from an umbilical cord; as such, a person of ordinary skill in the art realizes that a unit of cord blood obtained at parturition may be case-dependent and variable in volume (see National Marrow Donor Program website, www.marrow.org, and New York Blood Center website, www.nationalcordbloodprogram.org). However, to further prosecution, the claim was amended to clarify this matter, rendering the rejection moot.

CONCLUSIONS

Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. Accordingly, Applicants request that the Examiner issue a Notice of

Allowance for pending claims 1-8 and that the application be passed to issue. Applicants respectfully request that a Notice of Allowance of pending claims 1-8 be timely issued in this case.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided. The Commissioner is authorized to charge any deficiency in any patent application processing fees pursuant to 37 CFR §1.17, including extension of time fees pursuant to 37 CFR §1.17(a)-(d), associated with this communication and to credit any excess payment to Deposit Account No. 22-0261.

Respectfully submitted,

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